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**B661 B666 B67Y B670**  
**U1S S1313 S1321 S1325 S1328 S2410 S2415 S2416**

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**Other: BIOSIS, EPODOC, JAPIO, MEDLINE, WPI**

(54) Abstract Title: **Combination of a metal carbonyl compound and a guanylate cyclase stimulant or stabilizer for the therapeutic delivery of carbon monoxide**

(57) Metal carbonyls are used in combination with at least one guanylate cyclase stimulant/stabilizer to deliver CO having biological activity, for example vasodilatation and inhibition of platelet aggregation. The two components may be administered simultaneously or sequentially. A particularly useful combination is tricarbonylchloro(glycinato)ruthenium(II) and the guanylate cyclase stimulant/stabilizer 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl-indazole (YC-1).

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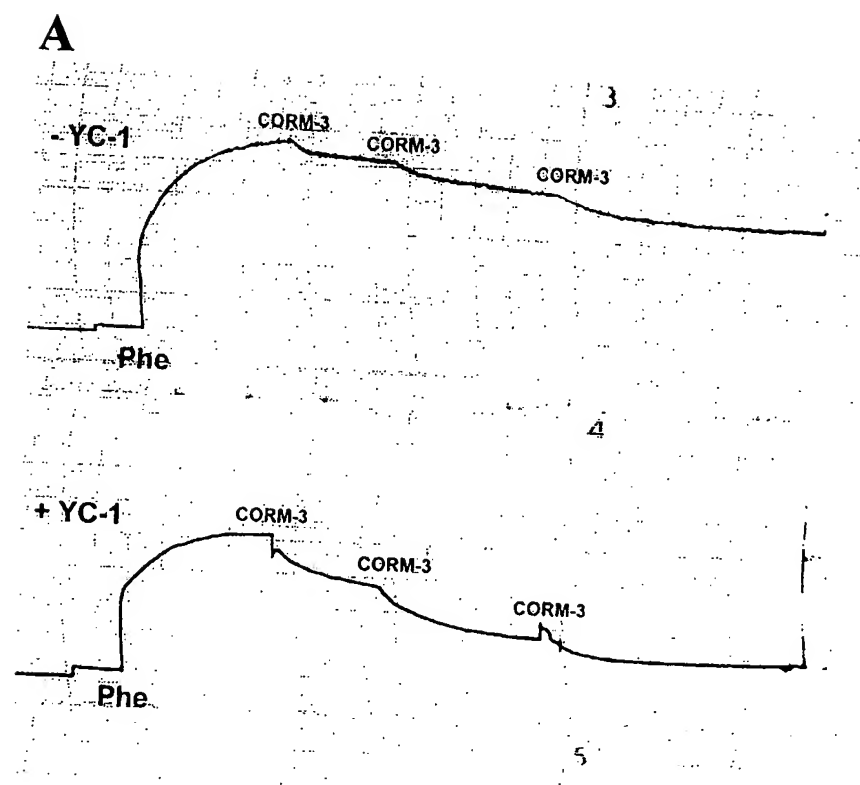


Figure 1A

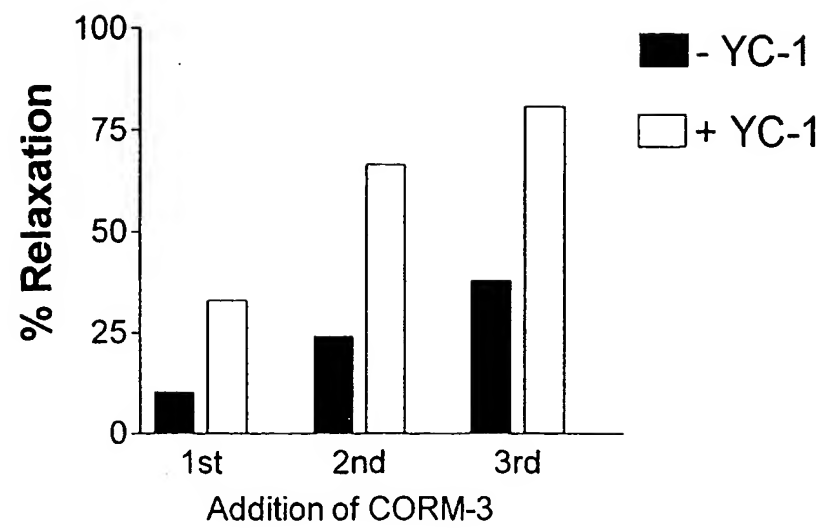
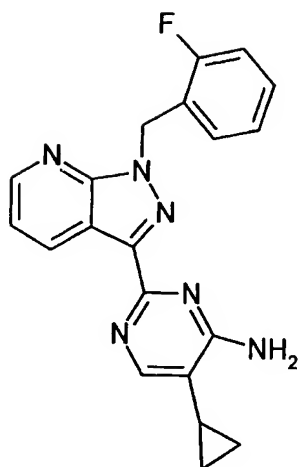
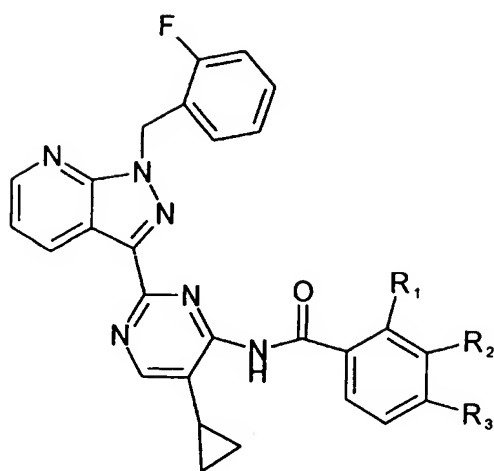
**B**

Figure 1B

3/9



BAY 41-2272



ortho PAL : R<sub>1</sub> = N<sub>3</sub> R<sub>2</sub> = H R<sub>3</sub> = H  
meta PAL : R<sub>1</sub> = H R<sub>2</sub> = N<sub>3</sub> R<sub>3</sub> = H  
para PAL : R<sub>1</sub> = H R<sub>2</sub> = H R<sub>3</sub> = N<sub>3</sub>

Figure 2

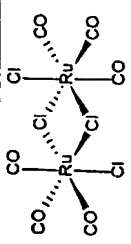
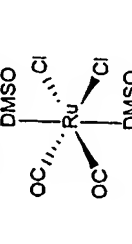
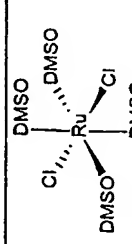
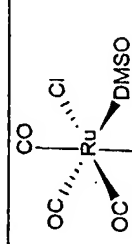
Compound	Structure	MW	CO Release (20 $\mu$ moles)				CO Release (40 $\mu$ moles)				NOTES
			0	10	20	30	0	10	20	30	
CO-RM-1		512	12.0 $\pm 3.0$	16.3 $\pm 4.0$	18.1 $\pm 4.3$	18.5 $\pm 4.8$	28.5 $\pm 0.4$	32.0 $\pm 0.2$	34.5 $\pm 0.5$	35.6 $\pm 0.4$	Soluble in DMSO
CO-RM-1a		384	7.2 $\pm 0.6$	8.6 $\pm 0.3$	8.0 $\pm 0.4$	7.5 $\pm 0.4$	16.9 $\pm 0.6$	18.4 $\pm 0.3$	17.3 $\pm 0.3$	16.7 $\pm 0.2$	Soluble in DMSO
Negative control		484	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Soluble in H <sub>2</sub> O
CO-RM-1b		334	6.4 $\pm 1.2$	7.3 $\pm 0.6$	8.2 $\pm 0.1$	8.7 $\pm 0.3$	11.7 $\pm 0.8$	13.7 $\pm 0.9$	14.0 $\pm 1.1$	14.4 $\pm 0.6$	Soluble in DMSO
CO-RM-10	$[Ru(CO)_2Cl_2]_n$	(228)	2.6 $\pm 0.6$	9.8 $\pm 0.3$	12.7 $\pm 0.1$	13.8 $\pm 0.9$	8.6 $\pm 0.7$	21.0 $\pm 1.1$	24.4 $\pm 1.0$	26.3 $\pm 1.2$	Soluble in DMSO

FIGURE 3A

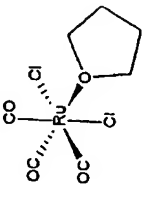
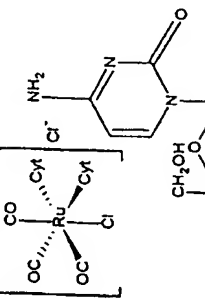
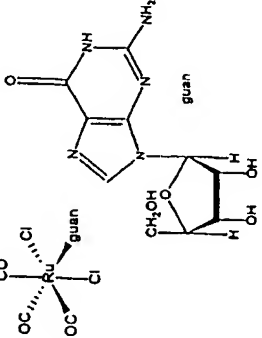
<b>CO-RM-11</b> Ligand: THF		328	5.6 ±0.6	5.9 ±0.6	6.2 ±1.1	6.2 ±1.2	10.9 ±0.2	12.3 ±0.4	13.3 ±0.4	13.7 ±0.2	Soluble in DMSO
<b>CO-RM-16</b> Ligand: Cytidine		742	N.D.	1.4 ±0.4	2.1 ±0.1	2.8 ±0.4	0.8 ±0.4	5.5 ±0.4	8.4 ±0.8	9.8 ±0.9	Soluble in H <sub>2</sub> O
<b>CO-RM-17</b> Ligand: Guanosine		539	5.9 ±0.1	8.2 ±0.4	8.5 ±0.3	8.6 ±0.4	11.5 ±0.4	15.0 ±0.4	15.6 ±0.4	16.2 ±0.3	Soluble in H <sub>2</sub> O

FIGURE 3B

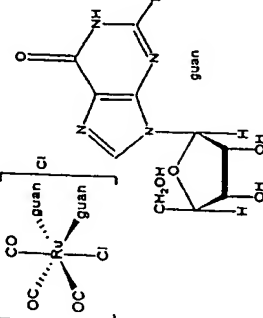
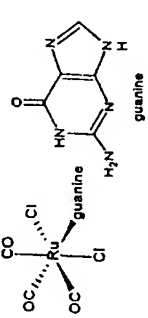
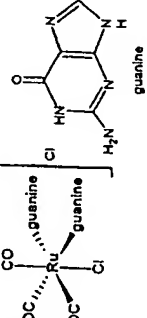
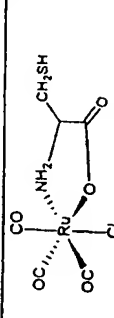
<b>CO-RM-18</b> Ligand: Guanosine		822	10.1 ±0.9	14.3 ±0.4	14.1 ±0.5	13.5 ±0.4	25.4 ±1.0	29.5 ±1.5	29.5 ±1.4	28.7 ±1.3	Soluble in H <sub>2</sub> O
<b>CO-RM-22</b> Ligand: Guanine		407	0.1 ±0.1	0.8 ±0.3	1.0 ±0.3	2.3 ±0.1	0.7 ±0.1	1.9 ±0.1	2.3 ±0.1	2.4 ±0.1	Soluble in H <sub>2</sub> O PPT
<b>CO-RM-23</b> Ligand: Guanine		558	1.2 ±0.1	1.3 ±0.2	1.3 ±0.1	1.0 ±0.2	2.7 ±0.3	2.7 ±0.3	2.7 ±0.4	2.3 ±0.2	Soluble in H <sub>2</sub> O PPT
<b>CO-RM-26</b> Ligand: Cysteine		340.5	0.6 ±0.1	1.9 ±0.1	2.3 ±0.2	2.4 ±0.2	1.9 ±0.2	3.7 ±0.1	5.1 ±0.1	5.2 ±0.1	Soluble in H <sub>2</sub> O

FIGURE 3C

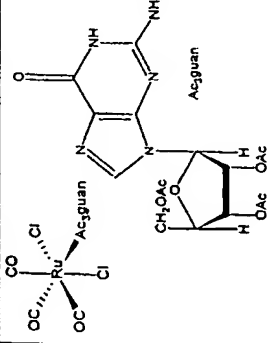
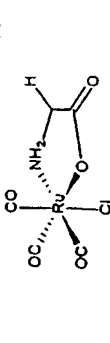
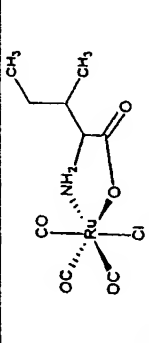
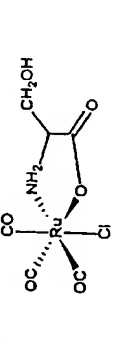
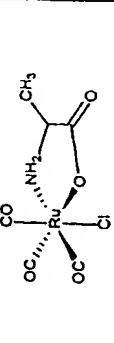
<b>CO-RM-29</b> Ligand: Triacetylguanosine		665	1.4 ±0.7	4.5 ±0.1	5.0 ±0.1	3.2 ±0.1	8.3 ±0.6	11.7 ±0.3	12.4 ±0.1	10.6 ±0.4	Soluble in H <sub>2</sub> O
<b>CO-RM-3</b> Ligand: Glycine		294.5	14.2 ±0.6	17.8 ±0.7	14.3 ±0.7	12.9 ±0.7	25.2 ±1.5	24.4 ±1.0	23.8 ±0.6	23.2 ±0.3	Soluble in H <sub>2</sub> O
<b>CO-RM-38</b> Ligand: Isoleucine		350.5	3.2 ±0.2	4.4 ±0.1	4.0 ±0.2	3.0 ±1.7	7.6 ±1.3	8.3 ±1.2	7.5 ±1.1	7.3 ±1.1	Soluble in H <sub>2</sub> O
<b>CO-RM-39</b> Ligand: Serine		324.5	11.0 ±0.3	12.8 ±0.9	11.4 ±1.1	10.8 ±0.7	24.2 ±1.5	24.6 ±1.4	22.0 ±1.0	21.9 ±1.2	Soluble in H <sub>2</sub> O
<b>CO-RM-40</b> Ligand: Alanine		308.5	9.1 ±1.1	11.9 ±0.4	11.1 ±0.3	11.0 ±0.2	20.2 ±0.6	21.3 ±0.9	19.9 ±0.9	19.6 ±0.9	Soluble in H <sub>2</sub> O

FIGURE 3D



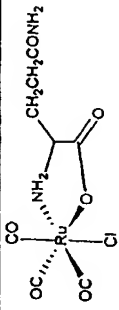
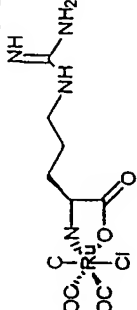
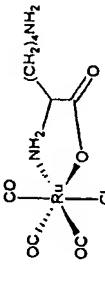
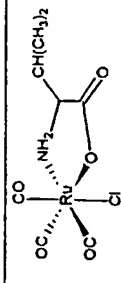
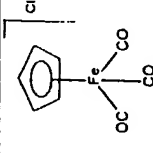
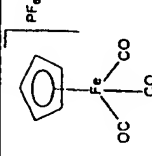
<b>CO-RM-42</b> Ligand: Glutamine		365.5	8.9 ±0.4	11.1 ±0.4	12.1 ±1.4	10.1 ±0.3	21.4 ±2.1	21.8 ±2.2	20.6 ±2.0	20.0 ±1.8	Soluble in H <sub>2</sub> O
<b>CO-RM-43</b> Ligand: Arginine		393.5	9.4 ±1.4	11.9 ±0.5	12.3 ±0.7	11.0 ±0.3	18.3 ±0.3	20.0 ±0.6	19.0 ±1.2	17.8 ±1.3	Soluble in H <sub>2</sub> O
<b>CO-RM-46</b> Ligand: Lysine		365.5	6.0 ±0.4	7.5 ±0.8	7.2 ±1.2	6.4 ±0.8	12.6 ±0.9	13.4 ±1.2	13.2 ±1.1	11.9 ±1.0	Soluble in H <sub>2</sub> O
<b>CO-RM-67</b> Ligand: L-valine		336.5	11.1 ±2.9	18.2 ±1.7	17.6 ±1.6	17.0 ±1.6	29.3 ±1.5	34.6 ±2.2	33.7 ±2.2	32.8 ±2.2	Soluble in H <sub>2</sub> O
<b>CO-RM-70</b>		240	0.5 ±0.2	0.9 ±0.1	2.2 ±0.2	2.7 ±0.3	0.9 ±0.1	2.0 ±0.2	4.9 ±0.2	6.3 ±0.3	Soluble in DMSO PPT
<b>CO-RM-71</b>		350	1.5 ±0.2	2.3 ±0.3	3.1 ±0.4	3.7 ±0.4	3.4 ±0.1	5.4 ±0.3	6.9 ±0.3	7.6 ±0.4	Soluble in DMSO PPT

FIGURE 3E

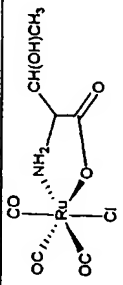
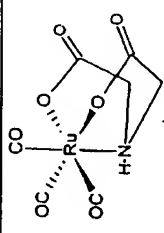
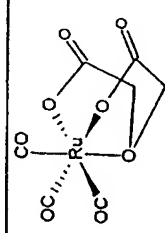
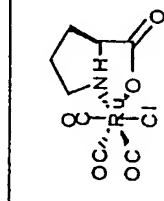
<b>CO-RM-74</b> Ligand: L-Threonine		338.5	15.7 ±1.2	17.5 ±2.0	16.5 ±2.3	14.8 ±2.2	33.3 ±0.2	33.4 ±0.1	32.7 ±0.2	31.4 ±0.1	Soluble in H <sub>2</sub> O
<b>CO-RM-97</b>		316	2.8 ±0.6	7.0 ±0.7	7.2 ±0.9	6.6 ±0.9	7.1 ±0.5	14.3 ±0.7	14.7 ±0.8	13.6 ±0.7	Soluble in H <sub>2</sub> O
<b>CO-RM-99</b>		317	4.6 ±0.6	8.1 ±0.2	7.3 ±0.3	5.5 ±0.3	11.5 ±0.2	16.6 ±0.2	16.0 ±0.9	14.0 ±0.2	Soluble in H <sub>2</sub> O
<b>CO-RM-H</b> Ligand: L-proline		335	1.4 ±0.3	4.7 ±0.6	6.2 ±0.8	6.3 ±0.7	4.2 ±0.4	9.9 ±0.2	12.5 ±0.1	13.0 ±0.1	Soluble in H <sub>2</sub> O

FIGURE 3F

Therapeutic Delivery of Carbon MonoxideFIELD OF THE INVENTION

5           The present invention relates to improved therapeutic delivery of carbon monoxide to humans and other mammals.

BACKGROUND OF THE INVENTION

10           The vasodilatory effects of nitric oxide (NO) and carbon monoxide (CO) gases have been known for some time (3). The L-arginine/NO synthase pathway present in the vascular endothelium plays a fundamental role in the control of vessel relaxation and arterial blood pressure  
15 in mammals (4). Increased generation of carbon monoxide (CO) following activation of the heme oxygenase-1 enzyme in the vascular tissue also results in suppression of acute hypertension *in vivo* (6) and prevention of vasoconstriction *ex vivo* (7).

20           Most recently, it has been reported that a series of transition metal carbonyls can be utilized as CO-releasing molecules (CO-RMs) in biological systems to elicit vasorelaxation and prevent increases in blood pressure (5).

25           Vascular relaxation by NO and CO appears to involve an increase in intracellular cyclic 3',5'-guanosine monophosphate (cGMP) levels through activation of a soluble heme-dependent guanylate cyclase (sGC) (3; 6; 7). However, it is known that CO is a poor stimulator of  
30 sGC in *in vitro* studies when compared to NO; the enzymatic activity of purified guanylate cyclase is increased 130-fold and 4.4-fold by its interaction with NO and CO, respectively (8).

Interestingly, data from the literature reveal that the catalytic rate of sGC can be substantially improved by the benzyl-indazole derivative 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl-indazole (YC-1). The mechanism  
5 underlying YC-1 action may be the stabilization of guanylate cyclase in its active conformation. It has also been suggested that YC-1 may stimulate production of guanylate cyclase.

Co-pending application PCT/GB02/02268 discloses  
10 various metal carbonyl compounds that can be used in the delivery of carbon monoxide to body cells and tissue. Some of the metal carbonyl compounds disclosed therein typically included a ligand other than CO. There was a statement that YC-1 may be used as a ligand.

15 One aim of the present invention is to provide an improved method of therapeutic delivery of carbon monoxide to the human or other mammal body.

#### SUMMARY OF THE INVENTION

20 As exemplified by the experimental data detailed below, the present inventors have found that metal carbonyl compounds can be used in combination with a guanylate cyclase stimulant or stabilizer to deliver CO to a physiological target so as to provide an improved  
25 physiological effect.

Accordingly, in a first aspect, the present invention provides a pharmaceutical preparation, for delivery of carbon monoxide to a physiological target, comprising a metal carbonyl compound or pharmaceutically  
30 acceptable salt thereof, a guanylate cyclase stimulant or stabilizer and at least one pharmaceutically acceptable carrier, wherein the metal carbonyl makes available CO suitable for physiological effect.

The preparation may contain the metal carbonyl and guanylate cyclase stimulant/stabilizer in a single composition or the two components may be formulated separately for simultaneous or sequential  
5 administration.

In a second aspect, the present invention provides a method of introducing CO to a mammal as a therapeutic agent comprising the step of administering a pharmaceutical preparation according to the first  
10 aspect.

In a third aspect, the present invention provides a method of introducing CO to a mammal as a therapeutic agent comprising:

- a) administering a metal carbonyl which makes  
15 available CO suitable for physiological effect; and
- b) administering a guanylate cyclase stimulant or stabiliser.

The metal carbonyl and guanylate cyclase stimulant/stabilizer may be administered simultaneously  
20 either in a single composition or in two separate compositions. Alternatively, the metal carbonyl and stimulant/stabilizer may be administered sequentially. Preferably, the stabilizer/stimulant is administered first followed by the metal carbonyl but this order may  
25 be reversed.

In a fourth aspect, the invention provides a kit comprising a) a metal carbonyl compound capable of making available CO suitable for physiological effect and b) a guanylate cyclase stimulant/stabilizer.

30 The two components may be for administration simultaneously or sequentially.

The various aspects of this invention are useful for treating a variety of body tissues. For example,

isolated organs e.g. extracorporeal organs or in situ organs isolated from the blood supply can be treated. The organ may be, for example, a circulatory organ, respiratory organ, urinary organ, digestive organ,  
5 reproductive organ, neurological organ, muscle or skin flap or an artificial organ containing viable cells. In particular, the organ may be a heart, lung, kidney or liver. However, the body tissue which is treatable are not limited and may be any human or mammal body tissue  
10 whether extracorporeal or in-situ in the animal body.

The various aspects of the present invention are used to provide a physiological effect, e.g. for stimulating neurotransmission or vasodilation, or for treatment of any of hypertension, such as acute,  
15 pulmonary and chronic hypertension, radiation damage, endotoxic shock, inflammation, inflammatory-related diseases such as asthma and rheumatoid arthritis, hyperoxia-induced injury, apoptosis, cancer, transplant rejection, arteriosclerosis, post-ischemic organ damage,  
20 myocardial infarction, angina, haemorrhagic shock, sepsis, penile erectile dysfunction, adult respiratory distress syndrome and inhibition of platelet aggregation.

The various aspects can also be used for perfusion,  
25 of a viable mammalian organ extracorporeally, e.g. during storage and/or transport of an organ for transplant surgery. For this purpose, the metal carbonyl is in dissolved form, preferably in an aqueous solution.

30 In the various aspects of the present invention, preferably, the metal carbonyl makes CO available by at least one of the following means:

1) CO derived by dissociation of the metal carbonyl is present in the composition in dissolved form;

2) on contact with a solvent or ligand the metal carbonyl releases CO;

3) on contact with a tissue, organ or cell the metal carbonyl releases CO;

4) on irradiation the metal carbonyl releases CO.

The most preferred metal carbonyls are water soluble metal carbonyls.

Certain metal carbonyl compounds are capable of releasing CO on contact with a suitable solvent. When the metal carbonyl component is to be administered in liquid form, this solvent may form a part of the component. Thus, the pharmaceutical preparation contains CO derived from the metal carbonyl in dissolved form. The conditions under which the carbonyl compound is dissolved in the solvent during preparation of the metal carbonyl component may be controlled such that the CO thus released is retained in solution. This may be facilitated where an equilibrium exists between the dissociated components and the undissociated carbonyl.

The dissociated components of the parent carbonyl may themselves be metal carbonyl complexes capable of releasing further CO. For example, when  $[\text{Ru}(\text{CO})_3\text{Cl}_2]_2$  is dissolved in DMSO, CO is liberated into solution, and a mixture of tri-carbonyl and di-carbonyl complexes is formed, and these themselves may be capable of releasing further CO.

Alternatively, the metal carbonyl component may not itself contain dissolved CO, but may be prepared such as to release CO on contact with a suitable solvent or medium. For example, the composition may contain a

metal carbonyl compound capable of releasing CO on contact with water, e.g. on contact with an aqueous physiological fluid, such as blood or lymph. The metal carbonyl compound may also release CO on contact with  
5 perfluorocarbon type blood substitute fluids or on contact with cardioplegic fluid.

Alternatively, the pharmaceutical composition may be intended to be dissolved in water prior to administration. Such metal carbonyl components may be  
10 prepared in solution or in solid form, such as in tablet form. If they are in solution form, they will typically be prepared in a solvent which does not support dissociation of the metal carbonyl compound, such that release of CO takes place only on contact with the  
15 appropriate substance.

Alternatively or additionally, release of CO from the carbonyl can be stimulated by reaction with a ligand in solution which for example replaces one of the ligands of the complex leading to loss of CO from the  
20 complex. The ligand may be one containing sulphur or nitrogen. Some metal carbonyls may release CO on contact with biological ligands such as glutathione or histidine.

As another alternative, the metal carbonyl  
25 component may contain a metal carbonyl compound which releases CO on contact with a tissue, organ or cell. It is known that certain metal carbonyl compounds do not release CO to solution but are nevertheless capable of releasing CO to physiological cellular materials or  
30 tissues, such as vascular endothelium. For example,  $[\text{Fe}(\text{SPh})_2(2,2'\text{-bipyridine})(\text{CO})_2]$  does not release CO to myoglobin in solution, but is nevertheless capable of promoting dilatation of pre-contracted aortic rings.



Without wishing to be limited by any particular theory,  
it is thought that CO may be released from such  
compounds as a result of an oxidation-reduction  
reaction, mediated by cellular components such as  
5 cytochromes.

However the invention is not limited to a redox  
reaction as a mechanism for CO release, since loss of at  
least a first CO from the complex may occur without  
redox.

10 As yet another alternative, the metal carbonyl  
component may contain a metal carbonyl compound which  
releases CO on irradiation. The compound may be  
irradiated prior to administration, for example to  
produce a solution of dissolved CO, or may be irradiated  
15 *in situ* after administration. It is contemplated that  
such compositions may be used to provide controlled,  
localised release of CO. For example a pharmaceutical  
composition of this type may be administered during  
surgery, and CO released specifically at a site in need  
20 thereof, e.g. to induce vasodilation, by localised  
irradiation by means of a laser or other radiant energy  
source, such as UV rays.

Typically the metal carbonyl components of the  
present invention release CO such as to make it  
25 available to a therapeutic target in dissolved form.  
However, in some circumstances CO may be released from a  
metal carbonyl directly to a non-solvent acceptor  
molecule.

Typically the metal carbonyl compound comprises a  
30 complex of a transition metal, preferably a transition  
metal from group 6 to 10 (in this specification the  
groups of the periodic table are numbered according to  
the IUPAC system from 1 to 18). The number of carbonyl

ligands is not limited, provided at least one carbonyl ligand is present. The preferred metals are transition metals of lower molecular weight, in particular Fe, Ru, Mn, Co, Ni, Mo and Rh. Two other metals which may be  
5 used are Pd and Pt. In the metal carbonyl complexes used in the invention, the metal is typically in a low oxidation state, i.e. 0, I or II. For the metals preferred, the oxidation states are typically not higher than  $\text{Fe}^{\text{II}}$ ,  $\text{Ru}^{\text{II}}$ ,  $\text{Mn}^{\text{I}}$ ,  $\text{Co}^{\text{II}}$  preferably  $\text{Co}^{\text{I}}$ ,  $\text{Rh}^{\text{III}}$  preferably  
10  $\text{Rh}^{\text{I}}$ ,  $\text{Ni}^{\text{II}}$ ,  $\text{Mo}^{\text{II}}$ . The metal is preferably not a radionuclide. Fe is one particularly suitable metal, since Fe is present in quantity in mammals.

The metal carbonyl compounds may be regarded as complexes, because they comprise CO groups coordinated  
15 to a metal centre. However the metal may be bonded to other groups by other than coordination bonds, e.g. by ionic or covalent bonds. Thus groups other than CO which form part of the metal carbonyl compound need not strictly be "ligands" in the sense of being coordinated  
20 to a metal centre via a lone electron pair, but will be referred to herein as "ligands" for ease of reference.

Thus, the ligands to the metal may all be carbonyl ligands, as e.g. in  $[\text{Mn}_2(\text{CO})_{10}]$ . Alternatively, the carbonyl compound may comprise at least one modulatory  
25 ligand. By this is meant a ligand which is not CO, but which modulates a particular property of the complex, such as the tendency to release CO, solubility, hydrophobicity, stability, electrochemical potential, etc. Thus suitable choices of ligand may be made in  
30 order to modulate the behaviour of the compound. For example it may be desirable to modulate the solubility of the compound in organic and/or aqueous solvents, its ability to cross cell membranes, its rate of release of

CO on contact with a particular solvent or cell type, or on irradiation, etc.

Such ligands are typically neutral or anionic ligands, such as halide, or derived from Lewis bases and having N, P, O, S or C as the coordinating atom(s). Preferred coordinating atoms are N, O and S. Examples include, but are not limited to, sulfoxides such as dimethylsulfoxide, natural and synthetic amino acids and their salts for example, glycine, cysteine, and proline, amines such as  $\text{NEt}_3$  and  $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$ , aromatic bases and their analogues, for example, bi-2,2'-pyridyl, indole, pyrimidine and cytidine, pyrroles such as biliverdin and bilirubin, thiols and thiolates such as  $\text{EtSH}$  and  $\text{PhSH}$ , chloride, bromide and iodide, carboxylates such as formate, acetate, and oxalate, ethers such as  $\text{Et}_2\text{O}$  and tetrahydrofuran, alcohols such as  $\text{EtOH}$ , and nitriles such as  $\text{MeCN}$ . Particularly preferred are coordinating ligands, such as amino acids, which render the carbonyl complex stable in aqueous solution. Other possible ligands are conjugated carbon groups, such as dienes. One class of ligands which can provide metal carbonyl compounds of use in this invention is cyclopentadienyl ( $\text{C}_5\text{H}_5$ ) and substituted cyclopentadienyl. The substituent group in substituted cyclopentadienyl may be for example an alkanol, an ether or an ester, e.g.  $-(\text{CH}_2)_n\text{OH}$  where n is 1 to 4, particularly  $-\text{CH}_2\text{OH}$ ,  $-(\text{CH}_2)_n\text{OR}$  where n is 1 to 4 and R is hydrocarbon preferably alkyl of 1 to 4 carbon atoms and  $-(\text{CH}_2)_n\text{OOCR}$  where n is 1 to 4 and R is hydrocarbon preferably alkyl of 1 to 4 carbon atoms. The preferred metal in such a cyclopentadienyl or substituted cyclopentadienyl carbonyl complex is Fe. Preferably the cyclopentadienyl carbonyl complex is

cationic, being associated with an anion such as chloride.

Thus the properties of pharmaceutical compositions of the present invention may be tailored as required by appropriate choice of metal centres and number and type  
5 of associated ligands in the metal carbonyl compound.

The metal carbonyl compound may further comprise a targeting moiety, to facilitate release of CO at an appropriate site. The targeting moiety is typically  
10 capable of binding a receptor on a particular target cell surface, in order to promote release of CO at the required site. The targeting moiety may be a part of a modulating ligand capable of binding to a receptor found on the surface of the target cells, or may be derived  
15 from another molecule, such as an antibody directed against a particular receptor, joined to the complex by a suitable linker.

The present invention also includes as the metal carbonyl component a compound of the formula  $M(CO)_x A_y$   
20 where x is at least one, y is at least one, M is a metal, A is an atom or group bonded to M by an ionic, covalent or coordination bond but is not CO, and, in the case where  $y > 1$ , each A may be the same or different, or a pharmaceutically acceptable salt of such a compound.  
25 Typically, M is a transition metal, particularly of groups 6 to 10, and A may be selected from neutral or anionic ligands, such as halide, or derived from Lewis bases and having N, P, O, S or C as the coordinating atom(s). Mono-, bi- or polydentate ligands may be used.  
30 More details of preferred metals and ligands are given above.

The carbonyl complex should be pharmaceutically acceptable, in particular non-toxic or of acceptable toxicity at the dosage levels envisaged.

5 The metal carbonyl component may be a compound of the formula

$M(CO)_x A_y B_z$  where

M is Fe, Co or Ru,

x is at least one,

y is at least one,

10 z is zero or at least one,

each A is a ligand other than CO and is monodentate or polydentate with respect to M and is selected from the amino acids

alanine  
15 arginine  
asparagine  
aspartic acid  
cysteine  
glutamic acid  
20 glutamine  
glycine  
histidine  
isoleucine  
leucine  
25 lysine  
methionine  
phenylalanine  
proline  
serine  
30 threonine  
tryptophan  
tyrosine  
valine

$[O(CH_2COO)_2]^{2-}$  and

$[NH(CH_2COO)_2]^{2-}$ , and

B is optional and is a ligand other than CO.

x is preferably 3, y is preferably 1 and z is  
5 preferably 1.

The term amino acid here used includes the species obtained by loss of the acidic hydrogen, such as glycinate.

B<sub>z</sub> represents one or more optional other ligands.  
10 There are no particular limitations on B, and ligands such as halides, e.g. chloride, bromide, iodide, and carboxylates, e.g. acetate may be used.

M is selected from Fe, Ru and Co. These metals are preferably in low oxidation states, as described above.

15 Use of the known iron compounds  $[Fe(SPh)_2(2,2'$ -bipyridine) $(CO)_2]$  and  $[Fe(SPh)_2(NH_2CH_2CH_2NH_2)(CO)_2]$  is also envisaged in this invention.

The guanylate cyclase stabilizer/stimulant compound may be any compound which stimulates production of  
20 guanylate cyclase or which stabilizes guanylate cyclase, in particular the active form of guanylate cyclase. A single compound can be used or a combination of compounds can be used either for simultaneous or sequential administration, i.e. the various aspects  
25 include/use at least one guanylate cyclase stimulant/stabilizer.

Examples include 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl-indazole (YC-1), 4 pyrimidinamine-5-cyclopropyl-2-[1-[(2-fluorophenyl)methyl]-1H-pyrazolo[3,4-b]pyridin-3-yl] (BAY 41-2272), BAY 50-6038 (ortho-PAL), BAY 51-9491 (meta PAL), and BAY 50-8364 (para PAL). The  
30 structures of ortho-, meta- and para- PAL are shown in Figure 2. These compounds have been found to bind to an

activation site on the guanylate cyclase (9) and any other compounds that similarly bind to the site may be useful as the guanylate cyclase stabilizer/ stimulant. Also useful are NO donors and 1-benzyl-3-(3'-ethoxycarbonyl)phenyl-indazole, 1-benzyl-3-(3'-hydroxymethyl)phenyl-indazole, 1-benzyl-3-(5'-diethylaminomethyl)-furyl-indazole, 1-benzyl-3-(5'-methoxymethyl)furyl-indazole, 1-benzyl-3-(5'-hydroxymethyl)furyl-6-methyl-indazole, 1-benzyl-3-(5'-hydroxymethyl)-furyl-indazol-benzyl-3-(5'-hydroxymethyl)-furyl-indazole, 1-benzyl-3-(5'-hydroxymethyl)-furyl-6-fluoro-indazole, 1-benzyl-3-(5'-hydroxymethyl)-furyl-6-methoxy-indazole, and 1-benzyl-3-(5'-hydroxymethyl)-furyl-5,6-methylenedioxindazole or pharmaceutically acceptable salts thereof.

The metal carbonyl component and/or guanylate cyclase stabilizer/stimulant component typically comprise a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere unduly with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, subcutaneous, nasal, intramuscular, intraperitoneal, or suppository routes.

Components/preparations for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant or a slow-release polymer. Liquid compositions/preparations generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological

saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. Pharmaceutically acceptable amounts of other solvents may also be included, in particular where they are required for dissolving the particular metal carbonyl compound contained in the composition. The composition may further comprise pharmaceutically acceptable additives such as suspending agents (e.g. sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g. lecithin or acacia); non-aqueous vehicles (e.g. almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); preservatives (e.g. methyl or propyl-p-hydroxybenzoates or sorbic acid); and energy sources (e.g. carbohydrates such as glucose, fats such as palmitate or amino acid).

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will typically be in the form of a parenterally acceptable solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required. Delivery systems for needle-free injection are also known, and compositions for use with such systems may be prepared accordingly.

Administration is preferably in a prophylactically effective amount or a therapeutically effective amount (as the case may be, although prophylaxis may be



considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

When formulating compositions/preparations according to the present invention, the toxicity of the active ingredient, stimulant/stabilizer and/or the solvent must be considered. The balance between medical benefit and toxicity should be taken into account. The dosages and formulations will typically be determined so that the medical benefit provided outweighs any risks due to the toxicity of the constituents. Examples include St Thomas Hospital solutions, Euro-Collins solutions, University of Wisconsin solutions, Celsior solutions, Ringer Lactate solutions, Bretschneider solutions and perflurocarbons.

The metal carbonyl compound and the stimulant/stabilizer can be formulated into a single composition that can be in any physical form. In this case, the components will be administered simultaneously. Alternatively, the components can be formulated into two compositions which can be administered simultaneously or sequentially.

Throughout this application, references to medical treatment are intended to include both human and veterinary treatment, and references to pharmaceutical compositions are accordingly intended to encompass  
5 compositions for use in human or veterinary treatment.

#### INTRODUCTION OF THE DRAWINGS

Experimental data illustrating the present invention will now be described by reference to the  
10 accompanying figures, in which:

Figure 1A shows vasodilatory effects of CORM-3 alone and in combination with YC-1;

Figure 1B shows percentage relaxation;

Figure 2 shows structures of ortho-, meta- and  
15 para- PAL; and

Figures 3A to F show carbon monoxide releasing molecules.

#### EMBODIMENTS OF THE INVENTION AND EXPERIMENTAL DATA

20

Stock solutions of CORM-3 (100 mM) were prepared by solubilizing the compound in distilled water prior to the experiment. Tricarbonyldichloro ruthenium(II) dimer ( $[\text{Ru}(\text{CO})_3\text{Cl}_2]_2$ ), 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl-indazole (YC-1) and all other reagents were purchased  
25 from Sigma-Aldrich (Poole, Dorset).

All data are expressed as mean  $\pm$  s.e.m. Differences between the groups analysed were assessed by the Student's two-tailed t-test, and an analysis of variance  
30 (ANOVA) was performed where more than two treatments were compared. Results were considered statistically significant at  $P < 0.05$ .

Syntheses

Synthetic methods for obtaining compounds of Figs. 3A to 3F were described in co-pending application PCT/GB02/02268 the entire content of which is  
 5 incorporated herein by reference.

Preparation of  $\text{Ru}(\text{CO})_3\text{Cl}(\text{NH}_2\text{CH}_2\text{CO}_2)$  [ $M_R$  294.5]

Glycine complex. Reference number: CORM-3

[ $\text{Ru}(\text{CO})_3\text{Cl}_2$ ]<sub>2</sub> (0.129g, 0.25 mmol) and glycine  
 10 (0.039g, 0.50 mmol) were placed under nitrogen in a round bottomed flask. Methanol (75 cm<sup>3</sup>) and sodium ethoxide (0.034g, 0.50 mmol) were added and the reaction stirred for 18 hours. The solvent was then removed under pressure and the yellow residue redissolved in  
 15 THF, filtered and excess 40-60 light petroleum added. The yellow solution was evaporated down to give a pale yellow solid (0.142g, 96%). CORM-3 was stored in closed vials at 4 C and used freshly on the day of the experiments.

20

Alternative, preferred preparation of $\text{Ru}(\text{CO})_3\text{Cl}(\text{NH}_2\text{CO}_2\text{CO}_2)$  [ $M_R$  294.6]

Glycine complex. Reference number: CORM-3

[ $\text{Ru}(\text{CO})_3\text{Cl}_2$ ]<sub>2</sub> (0.129g, 0.25 mmol) and glycine  
 25 (0.039g, 0.50 mmol) were placed under nitrogen in a round bottomed flask. Methanol (40 cm<sup>3</sup>) and sodium methoxide (0.5M solution in MeOH, 1.00 cm<sup>3</sup>, 0.50 mmol) were added and the reaction stirred for 18 hours. HCl (2.0M solution in diethyl ether) was added in small  
 30 aliquots until the IR band at 1987 cm<sup>-1</sup> in solution IR spectroscopy could no longer be detected. The solvent was then removed under reduced pressure and the yellow residue redissolved in THF, filtered and an excess of

40-60 light petroleum added. The resulting precipitate was isolated by pipetting off the mother liquor and drying under high vacuum. The same work up was repeated for the mother liquor once concentrated. The colour of  
5 the product varied between white and pale yellow and was produced in an average yield of 0.133g, (90%).

Preparation of isolated rat aortic rings and  
experimental protocol

10 The method for the preparation of isolated aortic rings has been previously described (5; 7). The thoracic aorta was isolated from Sprague-Dawley rats (350-450 g) and flushed with cold Krebs-Henseleit buffer (4°C, pH 7.4) containing (in mM): 118 NaCl, 4.7 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>,  
15 1.2 MgSO<sub>4</sub>·7H<sub>2</sub>O, 22 NaHCO<sub>3</sub>, 11 Glucose, 0.03 K<sup>+</sup>EDTA, 2.5 CaCl<sub>2</sub> and supplemented with 10 µM indomethacin. Each aorta was trimmed of adventitial tissue and ring sections (~3 mm length) were produced from the mid aortic segment. The rings were then mounted between two  
20 stainless steel hooks in 9-ml organ baths containing Krebs-Henseleit buffer which was maintained at 37 °C and continuously gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub>. One hook was attached to a Grass FT03 isometric force transducer whilst the other was anchored to a sledge for regulation  
25 of the resting tension of the aortic ring. The rings were initially equilibrated for 30 min under a resting tension of 2g which was previously determined to be optimal. Continuous recording of tension was made on a Grass 7D polygraph (Grass Instruments, Quincy, MA) in  
30 combination with a Biopac MP100 system using AcqKnowledge™ software (Linton Instruments, Norfolk, UK). Before each protocol was carried out, rings were

contracted with a standard dose of KCl (100 mM) in order to provide an internal reference and to control for variability in contractile responsiveness between tissues. The relaxation response to CORM-3 (25  $\mu$ M) in the presence or absence of YC-1 (5  $\mu$ M final concentration, 30 min pre-incubation) was assessed in aortic rings pre-contracted with phenylephrine (1  $\mu$ mol/L).

## 10 Results

Figure 1A shows the typical tracings of the vascular reactivity to phenylephrine and the vasodilatory effects of CORM-3 alone or in combination with YC-1. In the absence of YC-1, three sequential additions of CORM-3 (25  $\mu$ M each) to the pre-contracted ring elicited vasorelaxation (see top tracing). If the relaxation is expressed as a percentage of the maximal phenylephrine-mediated contraction, then we can calculate that CORM-3 produced a 10.3% relaxation after the first addition, 24.1% relaxation after the second addition and 38% after the third addition (Figure 1B). The presence of YC-1 in the organ bath amplified the observed vasodilatory effect mediated by CORM-3 (see bottom tracing, Figure 1A) and produced a 33% relaxation after the first addition of the CO carrier, 66.6% relaxation after the second addition and 80.9% after the third addition (Figure 1B). These data indicate that CO released by CORM-3 mediates a vasodilatory effect which can be further enhanced by addition of the sGC activator YC-1. In view of the fact that increased cGMP levels by YC-1 in the presence of CO led to complete inhibition of platelet aggregation (1), the results presented here point to the potential therapeutic use of CORM-3 in

combination with YC-1 in those pathophysiological conditions characterized by increased platelet aggregation.

5 While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth above are considered to be  
10 illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention.

## References:

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**CLAIMS:**

1. A pharmaceutical preparation, for delivery of carbon monoxide to a physiological target, comprising a metal carbonyl compound or pharmaceutically acceptable salt thereof, a guanylate cyclase stimulant or stabilizer and at least one pharmaceutically acceptable carrier, wherein the metal carbonyl makes available CO suitable for physiological effect.
2. A pharmaceutical preparation according to claim 1 wherein said metal carbonyl compound makes CO available by at least one of the following means:
- 1) CO derived by dissociation of the metal carbonyl is present in the composition in dissolved form;
  - 2) on contact with a solvent or ligand the metal carbonyl releases CO;
  - 3) on contact with a tissue, organ or cell the metal carbonyl releases CO;
  - 4) on irradiation the metal carbonyl releases CO.
3. A pharmaceutical preparation according to claim 1 or claim 2 wherein said metal carbonyl compound and said guanylate cyclase stimulant/stabilizer are combined in a single composition.
4. A pharmaceutical preparation according to claim 1 or claim 2 wherein said metal carbonyl compound and said guanylate cyclase stabilizer/stimulant are in separate compositions for administration simultaneously or sequentially.

5. A pharmaceutical preparation according to any one of the preceding claims wherein the metal carbonyl compound has the formula  $M(CO)_x A_y$  where x is at least one, y is at least one, M is a metal, the or each A is an atom or group bonded to M by an ionic, covalent or coordination bond but is not CO, and in the case where  $y > 1$  each A may be the same or different, or a pharmaceutically acceptable salt of such a compound.
6. A pharmaceutical preparation according to claim 5 wherein M is a transition metal.
7. A pharmaceutical preparation according to claim 5 or claim 6, wherein A is selected from neutral or anionic ligands, such as halide, or derived from Lewis bases and having N, P, O, S or C as the coordinating atom(s).
8. A pharmaceutical preparation according to any one of claims 1 to 4 wherein the metal carbonyl compound has the formula
- $M(CO)_x A_y B_z$  where
- M is Fe, Co or Ru,
- x is at least one,
- y is at least one,
- z is zero or at least one,
- each A is a ligand other than CO and is monodentate or polydentate with respect to M and is selected from the amino acids
- alanine
- arginine
- asparagine
- aspartic acid

cysteine  
 glutamic acid  
 glutamine  
 glycine  
 5 histidine  
 isoleucine  
 leucine  
 lysine  
 methionine  
 10 phenylalanine  
 proline  
 serine  
 threonine  
 tryptophan  
 15 tyrosine  
 valine  
 $[\text{O}(\text{CH}_2\text{COO})_2]^{2-}$  and  
 $[\text{NH}(\text{CH}_2\text{COO})_2]^{2-}$ , and  
 B is optional and is a ligand other than CO.

20

9. A pharmaceutical preparation according to any one of the preceding claims wherein the guanylate cyclase stimulant/stabilizer is YC-1.
- 25 10. A pharmaceutical composition according to any one of the preceding claims adapted for delivery by an oral, intravenous, subcutaneous, nasal, inhalatory, intramuscular, intraperitoneal or suppository route.
- 30 11. A method of introducing CO to a mammal as a therapeutic agent comprising the step of administering a pharmaceutical preparation according to any one of the preceding claims.

12. A method of introducing CO to a mammal as a therapeutic agent comprising:

- 5       a) administering a metal carbonyl which makes available CO suitable for physiological effect; and  
      b) administering a guanylate cyclase stimulant or stabiliser.

13. A method according to claim 12 wherein said metal carbonyl compound makes CO available by at least one of the following means:

- 1) CO derived by dissociation of the metal carbonyl is present in the composition in dissolved form;  
15       2) on contact with a solvent or ligand the metal carbonyl releases CO;  
      3) on contact with a tissue, organ or cell the metal carbonyl releases CO;  
      4) on irradiation the metal carbonyl releases CO.

20

14. A method according to claim 12 or claim 13 wherein the steps of administering the metal carbonyl and guanylate cyclase stimulant/stabilizer are simultaneous.

25   15. A method according to claim 12 or claim 13 wherein the steps of administering the metal carbonyl and guanylate cyclase stimulant/stabilizer are sequential.

30   16. A method according to any one of claims 12 to 15 wherein the metal carbonyl compound has the formula  $M(CO)_x A_y$  where x is at least one, y is at least one, M is a metal, the or each A is an atom or group bonded to M by an ionic, covalent or coordination bond but is not

CO, and in the case where  $y > 1$  each A may be the same or different, or a pharmaceutically acceptable salt of such a compound.

- 5 17. A method according to claim 16 wherein M is a transition metal.
18. A method according to claim 16 or claim 17, wherein A is selected from neutral or anionic ligands, such as
- 10 halide, or derived from Lewis bases and having N, P, O, S or C as the coordinating atom(s).
19. A method according to any one of claims 12 to 15 wherein the metal carbonyl compound has the formula
- 15  $M(CO)_x A_y B_z$  where  
M is Fe, Co or Ru,  
x is at least one,  
y is at least one,  
z is zero or at least one,
- 20 each A is a ligand other than CO and is monodentate or polydentate with respect to M and is selected from the amino acids
- alanine
- arginine
- 25 asparagine
- aspartic acid
- cysteine
- glutamic acid
- glutamine
- 30 glycine
- histidine
- isoleucine
- leucine

- lysine  
methionine  
phenylalanine  
proline  
5 serine  
threonine  
tryptophan  
tyrosine  
valine  
10  $[O(CH_2COO)_2]^{2-}$  and  
 $[NH(CH_2COO)_2]^{2-}$ , and  
B is optional and is a ligand other than CO.

20. A method according to any one of claims 12 to 19  
15 wherein the guanylate cyclase stimulant/stabilizer is  
YC-1.

21. A method according to any one of claims 12 to 20  
wherein the metal carbonyl compound and/or the guanylate  
20 cyclase stabilizer/stimulant is administered by an oral,  
intravenous, subcutaneous, nasal, inhalatory,  
intramuscular, intraperitoneal or suppository route.

22. A method according to any one of claims 11 to 21  
25 wherein the metal carbonyl and guanylate cyclase  
stimulant/stabilizer are administered to an  
extracorporeal body organ.

23. A method according to any one of claims 11 to  
30 22 where the administration is for the stimulation of  
vasodilation, or for treatment of any of hypertension,  
such as acute, pulmonary and chronic hypertension,  
radiation damage, endotoxic shock, inflammation,

inflammatory-related diseases such as asthma and  
rheumatoid arthritis, hyperoxia-induced injury,  
apoptosis, cancer, transplant rejection,  
arteriosclerosis, post-ischemic organ damage, myocardial  
5 infarction, angina, haemorrhagic shock, sepsis, penile  
erectile dysfunction, adult respiratory distress  
syndrome and inhibition of platelet aggregation.

24. A kit comprising a) a metal carbonyl compound  
10 capable of making available CO suitable for  
physiological effect; and b) a guanylate cyclase  
stimulant/stabilizer.

25. A kit according to claim 24 wherein said metal  
15 carbonyl compound makes CO available by at least one of  
the following means:

- 1) CO derived by dissociation of the metal  
carbonyl is present in the composition in dissolved  
form;
- 20 2) on contact with a solvent or ligand the metal  
carbonyl releases CO;
- 3) on contact with a tissue, organ or cell the  
metal carbonyl releases CO;
- 25 4) on irradiation the metal carbonyl releases CO.

26. A kit according to claim 24 or claim 25 wherein  
said metal carbonyl compound and said guanylate cyclase  
stabilizer/stimulant are in separate compositions for  
administration simultaneously or sequentially.

30 27. A kit according to any one of claims 24 to 26  
wherein the metal carbonyl compound has the formula  
 $M(CO)_x A_y$  where x is at least one, y is at least one, M is

a metal, the or each A is an atom or group bonded to M by an ionic, covalent or coordination bond but is not CO, and in the case where  $y > 1$  each A may be the same or different, or a pharmaceutically acceptable salt of such a compound.

28. A kit according to claim 27 wherein M is a transition metal.

29. A kit according to claim 27 or claim 28, wherein A is selected from neutral or anionic ligands, such as halide, or derived from Lewis bases and having N, P, O, S or C as the coordinating atom(s).

30. A kit according to any one of claims 24 to 26 wherein the metal carbonyl compound has the formula

$M(CO)_x A_y B_z$  where

M is Fe, Co or Ru,

x is at least one,

y is at least one,

z is zero or at least one,

each A is a ligand other than CO and is monodentate or polydentate with respect to M and is selected from the amino acids

alanine

arginine

asparagine

aspartic acid

cysteine

glutamic acid

glutamine

glycine

histidine



isoleucine  
leucine  
lysine  
methionine  
5 phenylalanine  
proline  
serine  
threonine  
tryptophan  
10 tyrosine  
valine

$[O(CH_2COO)_2]^{2-}$  and

$[NH(CH_2COO)_2]^{2-}$ , and

B is optional and is a ligand other than CO.

15

31. A kit according to any one of claims 24 to 30 wherein the guanylate cyclase stimulant/stabilizer is YC-1.

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32. A kit according to any one of claims 24 to 31 wherein the metal carbonyl and/or the guanylate cyclase stabilizer/stimulant is adapted for delivery by an oral, intravenous, subcutaneous, nasal, inhalatory, intramuscular, intraperitoneal or suppository route.

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Application No: GB 0227135.1  
Claims searched: 1-32

Examiner: Stephen Quick  
Date of search: 22 April 2003

## Patents Act 1977 : Search Report under Section 17

### Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A,E	-	WO 02/092075 A2 (NORTHWICK PARK INSTITUTE FOR MEDICAL RESEARCH & UNIVERSITY OF SHEFFIELD) 21.11.2002, see page 12 lines 18-27; acknowledged in this application as PCT/GB02/02268
A	-	MEDLINE Abstract Accession No NLM11916917 & Diabetes, 2002, Vol. 51(4), pages 994-999, see abstract

### Categories:

X Document indicating lack of novelty or inventive step	A Document indicating technological background and/or state of the art.
Y Document indicating lack of inventive step if combined with one or more other documents of same category.	P Document published on or after the declared priority date but before the filing date of this invention.
& Member of the same patent family	E Patent document published on or after, but with priority date earlier than, the filing date of this application.

### Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC<sup>V</sup>:

A5B

Worldwide search of patent documents classified in the following areas of the IPC<sup>7</sup>:

A61K

The following online and other databases have been used in the preparation of this search report:

BIOSIS, EPODOC, JAPIO, MEDLINE, WPI